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The electrophysiology of feeding circuits

Erin E. Jobst, Pablo J. Enriori and Michael A. Cowley

Division of Neuroscience, Oregon National Primate Research Center, Oregon Health Sciences University, 505 NW 185th Avenue, Beaverton, OR 97006, USA

Obesity is quickly becoming one of the most common and debilitating disorders of the developed world. More than 60% of American adults are now overweight or obese, predisposing them to a host of chronic diseases. To understand the etiology of obesity, and to discover new therapies for obesity, we must understand the components of energy balance. In simple terms, energy intake (feeding) must equal energy expenditure (physical activity, basal metabolism and adaptive thermogenesis) for body weight homeostasis. To maintain homeostasis, neurocircuitry must sense both immediate nutritional status and the amount of energy stored in adipose tissue, and must be able to provide appropriate output to balance energy intake and energy expenditure. The brain receives various signals that carry nformation about nutritional and metabolic status including neuropeptide PYY_{3-36}, ghrelin, cholecystokinin, leptin, glucose and insulin. Circulating satiety signals access the brain either by 'leakage' across circumventricular organs or transport across the blood–brain barrier. Signals can also activate sensory vagal terminals that innervate the whole gastrointestinal tract.

By whatever means central neurons receive feedback regarding feeding status (and this is the subject of continued debate), it is clear that the effects of peripheral satiety signals are mediated by specific signal transduction systems in identifiable areas of the brain that are known to control food intake and body weight. In particular, the arcuate nucleus of hypothalamus (ARH) is a crucial integrative center for modulating food intake [1,2]. The ARH contains at least two key populations of neurons that have opposite actions on food intake. One population expresses anorexigenic (appetite-suppressing) peptides, cocaine- and amphetamine-regulated transcript (CART) and alpha-melanocyte-stimulating hormone (α-MSH; derived from the proopiomelanocortin (POMC) precursor). The other population expresses the orexigenic (appetite-stimulating) peptides neuropeptide Y (NPY) and agouti-related peptide (AgRP) [3]. Neurons in the ARH subsequently innervate various second order hypothalamic targets (Figure 1) that express melanocortin-4 (MC4) and NPY receptors [4].

Corresponding author: Michael A. Cowley (cowleym@ohsu.edu).
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Several excellent reviews have been written on central feeding circuits [5–7]. These have primarily focused on how peripheral feeding-related signals engage hypothalamic circuits and alter the levels of various hypothalamic neuropeptides (e.g. NPY and melanocortins) or monoamine neurotransmitters (e.g. serotonin, noradrenaline and possibly dopamine) in the central nervous system (CNS). Most research investigating the central control of appetite focuses on the hypothalamus. We review the neural circuits within the hypothalamus and their connections to relevant brainstem circuits.

Importance of electrophysiology in understanding central feeding circuits

Many studies have evaluated the functional activity of central feeding circuits by studying the mRNA or protein expression of c-Fos or other immediate early gene products; for example, an increase in c-Fos mRNA and/or protein in individual neurons has been used as a marker of neuronal activation [8]. Although such changes in the expression of immediate early gene products suggest that the functional output of neural circuitry has changed, they do not provide direct information about neuronal activity. For example, several peripheral signals, notably leptin, inhibit neuronal activity, a response that cannot be easily detected by analyzing c-Fos levels. Neuronal activity can also be inferred by analyzing changes in the concentration of intracellular Ca^{2+} detected by fluorescent dyes that alter their spectral response after binding to Ca^{2+} systems. Although these imaging systems provide some valuable data about neuronal activity, they generally lack single-cell resolution.

A crucial component that is missing in both mRNA and Ca^{2+} imaging studies is the activity 'pattern' of individual neurons. Indeed, the activity pattern of individual neurons might be essential in determining neurotransmitter release. Bursts of action potentials, which more effectively stimulate neuropeptide release [9], cannot be distinguished from fast, repetitive firing in c-Fos studies. Likewise, changes in the regional levels of anorexigenic or orexigenic neuropeptide mRNA might not be related to changes in the synaptic release of these neuropeptides. Ultimately, to understand how feeding-related signals relay appropriate information to various brain regions, the direct effect of such signals on neuronal activity must be assessed.

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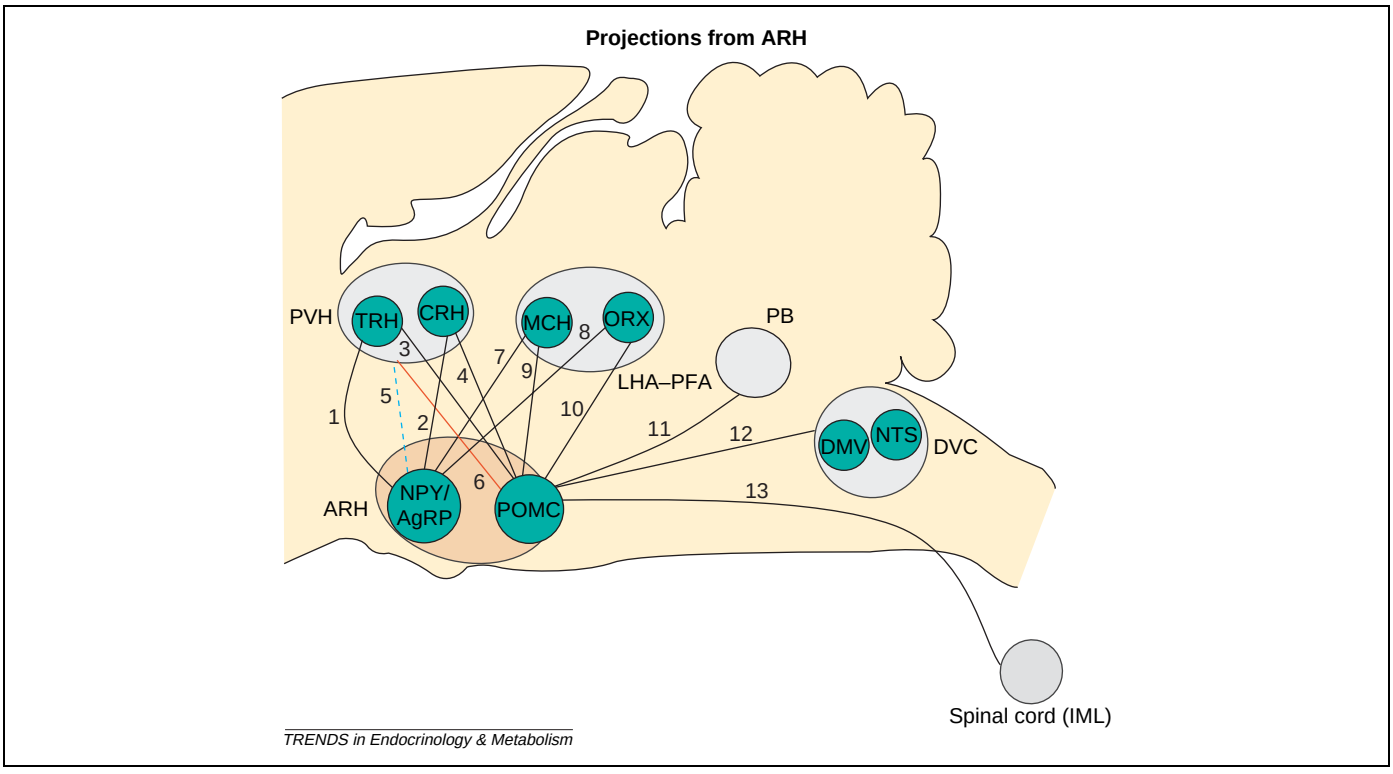

Figure 1. Projections from the arcuate nucleus of the hypothalamus (ARH). Shown are projections from the ARH to various hypothalamic nuclei including the paraventricular nucleus of the hypothalamus (PVH), lateral hypothalamic area (LHA) and ventromedial nucleus of the hypothalamus (VMH), and to extrahypothalamic areas such as the parabrachial nucleus (PB), dorsal vagal complex (DVC) and sympathetic preganglionic neurons in the spinal cord. Black lines indicate projections derived from anatomical studies, the broken cyan line shows inhibitory inputs derived from physiological studies, and the red line shows stimulatory inputs. Numbers to the left of each line refer to supporting references.Neuropeptide Y and agouti-related protein (NPY/AgRP) and proopiomelanocortin (POMC) terminals converge on neurosecretory parvocellular neurons in the PVH that express either thyrotropin-releasing hormone (TRH) or corticotropin-releasing hormone (CRH) (1) [104,105,106], (2) [107], (3) [103], (4) [103]. Neurons within the PVH can detect and integrate orexigenic and anorexigenic signals from the ARH (5 and 6) [55]. ARH neurons project to the LHA, a region containing the orexigenic hormones melanin-concentrating hormone (MCH) and orexin (ORX). NPY/AgRP terminals lie in close proximity to perikarya and processes of both MCH and ORX neurons (7) [95,108], (8) [68,95,107,108]. POMC neurons also innervate MCH and ORX neurons (9) [68,108], (10) [68,108]. NPY/AgRP and POMC neurons have monosynaptic connections with the PB and DVC, brainstem areas involved in food intake control (11) [109], (12) [110]. The DVC serves as the hub of a central neural network that provides parasympathetic control over the gastrointestinal tract and coordinates digestive functions. Direct melanocortinergic projections from the ARH also project to the interomediolateral (IML) column, which contains the sympathetic preganglionic neurons in the spinal cord (13) [56,108]. This pathway may mediate leptin effects on sympathetic responses. Regulation of ARH NPY/AgRP and POMC neurons by leptin and the projections of these cells to the LHA, PVH, and pools of autonomic preganglionic neurons in the medulla and spinal cord are likely to be critical in the regulating endocrine, autonomic, and behavioral effects of leptin. Abbreviations: DMV, dorsal motor nucleus of the vagus; NTS, nucleus of the solitary tract; PFA, perifornical area.

Most of the electrophysiological data that we review here are from recordings of individual neurons made after the application of feeding-related signals to brain slices. This type of preparation allows an assessment of the effect of various signals on individual neurons in systems in which at least part of the neural circuitry is intact. By combining data from slice preparations with *in vivo* studies, our goal is to build a working 'circuit diagram' of feeding pathways. For brevity, we have chosen to review the electrophysiological effects of only a few feeding-related signals on neurons in various nuclei in the brain. Inclusion of these factors does not imply their preeminence in feeding circuitry; similarly, the exclusion of other factors does not suggest that those factors are insignificant. Rather, we have selected the feeding-related signals discussed below because we know more about them and because a synthesis of their actions is overdue.

Electrophysiology of leptin

In addition to regulating long-term energy balance by its genomic effects on NPY/AgRP and POMC neurons, leptin has rapid electrophysiological effects on these two neuronal populations (Box 1). Because POMC and NPY/AgRP neurons in the ARH show dense expression of the leptin receptor [10,11] and are the source of potent feeding-related neuropeptides, our group [2,12] has carried out electrophysiological studies on brain slice preparations taken from transgenic mice that have been engineered to express fluorescent proteins in these specific cell types. Using these models, we have shown that leptin rapidly increases the frequency of action potentials in POMC neurons by two distinct mechanisms: first, leptin causes direct depolarization through a nonspecific cation channel; and second, leptin causes a decrease in inhibitory γ-aminobutyric acid (GABA)-mediated tone onto POMC cells by local orexigenic neurons expressing NPY and GABA (Figure 2) [2]. The second mechanism – that is, activation of POMC neurons by 'disinhibition' of local GABAergic tone – recurs as a common mechanism of modulating neuronal activity that is used by several other feeding-related signals.

In contrast to its rapid depolarization of POMC neurons, leptin hyperpolarizes and decreases the firing rate of NPY/AgRP neurons ([12,13]; and E.E. Jobst and M.A. Cowley, unpublished). Although definitive evaluation of the signal transduction pathway of leptin in NPY/AgRP

Box 1. Peripheral signals regulating long-term body weight homeostasis

Leptin

Substantial evidence has shown that leptin, a circulating anorexigenic hormone produced by white adipocytes [61], has a fundamental role in a neuroendocrine feedback loop involved in maintaining energy homeostasis [62]. In obese leptin-deficient mice, exogenous administration of leptin effectively reduces hyperphagia and obesity [63]. Conversely, obese mice that are deficient in the signaling form of the leptin receptor do not respond to leptin [63,64].

Leptin receptors (ObRb) are highly expressed in regions of the hypothalamus that mediate energy homeostasis [65,66]. To access these receptors, peripheral leptin is transported across the blood–brain barrier to reach areas distal to circumventricular organs [25]. In the arcuate nucleus of hypothalamus (ARH), leptin exerts some of its effects by acting through ObRb receptors on at least two distinct populations of neurons. Leptin reduces the mRNA expression of two potent orexigens, neuropeptide Y (NPY) [66] and agouti-related protein (AgRP) [67]. By contrast, leptin increases the expression of proopiomelanocortin (POMC) mRNA, which might promote the release of the peptide α-melanocyte-stimulating hormone (α-MSH), a potent anorexigen at central melanocortin-4 (MC-4) receptors [68].

Insulin

Insulin, a pancreatic hormone that is essential for stimulating glucose uptake and metabolism in peripheral tissues, was the first identified circulating signal proposed to be involved in energy homeostasis [69]. Central administration of insulin decreases food intake and body weight, and insulin receptors are concentrated in brain regions that are involved in controlling the intake of food (reviewed in Ref. [15]).

neurons in the ARH awaits further investigation, it has been reported that leptin hyperpolarizes a subset of ARH neurons by activating ATP-sensitive K^+ potassium (K_{ATP}) channels, similar to the mechanism reported for the action of insulin on hypothalamic neurons [14].

On the basis of these electrophysiology results, leptin can increase the release of anorexigenic peptides (CART and α-MSH) by upregulating the activity of POMC neurons, and can decrease the release of orexigenic peptides (NPY and AgRP) by downregulating the activity of NPY/AgRP neurons. The direct effects of leptin on ARH neuronal activity represent key mechanisms by which leptin inhibits food intake and increases energy expenditure. Selective deletion or inhibition of leptin receptors on ARH neurons will enable use to determine precisely how much of the effect of leptin on energy homeostasis is due to its actions on ARH neurons.

Electrophysiology of insulin

Comparatively little electrophysiology has been done on the mechanism of insulin action in the brain, even though both physiological and anatomical evidence show that the activation of central insulin receptors decreases food intake and body weight (Box 1) (reviewed in Ref. [15]). In ARH and ventromedial nucleus of hypothalamus (VMH) nuclei, physiological levels of insulin hyperpolarize and decrease the spontaneous firing rate of a subpopulation of neurons by opening K_{ATP} channels [16]. Insulin has a similar effect on hypothalamic neurons from *lean* Zucker rats that carry a missense mutation of the leptin receptor, rendering these animals insensitive to leptin;

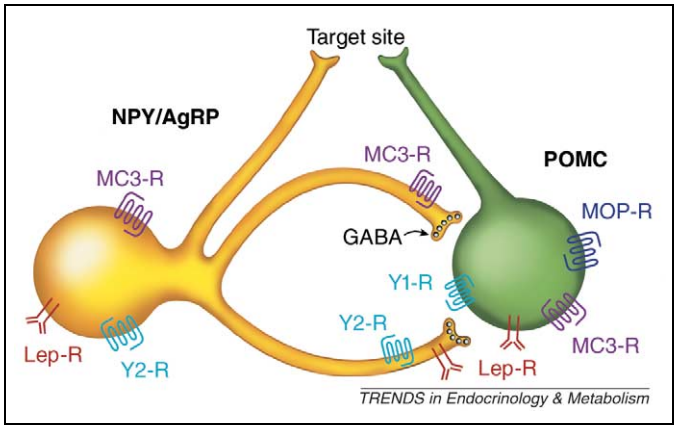

Figure 2. Model of leptin regulation of neurons expressing neuropeptide Y and agouti-related protein (NPY/AgRP neurons) and proopiomelanocortin (POMC neurons) in the arcuate nucleus of hypothalamus (ARH). Leptin directly depolarizes POMC neurons, simultaneously hyperpolarizes the somata of GABAergic NPY/AgRP neurons, and diminishes release from these terminals. This diminished release of GABA disinhibits POMC neurons. The direct and indirect effects of leptin together result in an activation of POMC neurons and an increase in the frequency of action potentials. Abbreviations: ARH, arcuate nucleus of hypothalamus; GABA, gamma-aminobutyric acid; Lep-R, leptin receptor; MC3-R, melanocortin-3 receptor; MOP-R, mu-opioid receptor; NPY/AgRP, neuropeptide Y/ agouti-related protein; POMC, proopiomelanocortin; Y1-R, NPY Y1 receptor; Y2-R, NPY Y2 receptor.

however, it has no effects on the membrane of VMH or ARH neurons from *obese* Zucker rats [16].

These observations highlight the probability of cross-talk between signaling events downstream of insulin receptors and leptin receptors in hypothalamic neurons involved in energy homeostasis. Indeed, research has indicated that phosphatidylinositol 3-kinase has a role in both leptin and insulin signaling [1]. It is tantalizing to propose that cross-desensitization occurs between the insulin and leptin pathways – in other words, insulin resistance at the intracellular signaling levels causes a relative resistance to leptin, thereby enabling the body to tolerate new adiposity. Future investigations need to tease apart the specificity of each pathway, the potential points of cross-talk and, in particular, how such interactions vary in pathophysiological conditions such as diabetes and obesity.

Electrophysiology of ghrelin

Ghrelin in the ARH

Although evidence indicates that circulating ghrelin might activate vagal afferents and subsequent brainstem pathways [17,18], its role in feeding regulation seems to be predominantly mediated by its actions in the ARH (Box 2) [19]. In hypothalamic slices containing the ARH, ghrelin dose-dependently stimulates electrical activity in most ARH neurons that are inhibited by leptin [12,13].

Recently, we and others have shown that ghrelin directly activates NPY/AgRP neurons [12,13]. Bath application of ghrelin was found to increase the spontaneous action potential frequency of NPY/AgRP neurons by 300%. Conversely, the same dose of ghrelin caused a 50% decrease in the spontaneous activity of POMC neurons. The latter effect on POMC neurons was abolished by the blockade of Y1 (the NPY receptor subtype on POMC neurons) and $GABA_A$ receptors, demonstrating that the inhibitory effect of ghrelin on POMC neurons is dependent

Box 2. Peripheral signals regulating short-term body weight homeostasis

Ghrelin

Ghrelin was the first peripheral orexigenic signal to be identified [70]. Ghrelin is the endogenous ligand of the growth hormone secretagogue receptor (GHS-R), which is densely expressed in pituitary and hypothalamic nuclei [71]. Although ghrelin is expressed in almost all tissues, its expression is highest in the stomach, where its secretion from A-like cells is upregulated during fasting and hypoglycemia [72]. Early studies have also detected ghrelin expression in the brain [70]. So far, however, the specific CNS expression pattern of ghrelin has been characterized only in the telencephalon and hypothalamus [12].

Whereas leptin inhibits food intake and increases energy expenditure, ghrelin produces a positive energy balance by promoting food intake and decreasing energy expenditure [21,73]. Expression and secretion of ghrelin decrease with feeding and obesity [74,75] and increase with caloric restriction and weight loss [76,77]. Accumulating evidence also suggests that a decrease in circulating ghrelin is an important factor in the long-term success of weight control after some bariatric surgical procedures [78].

PYY

Neuropeptide PYY comprises 36 amino acids and is released from enteroendocrine L cells that line the distal small bowel and colon [79]. About 40% of PYY is enzymatically cleaved in the gastrointestinal tract, resulting in a shortened form of PYY containing 34 amino acids termed $PYY_{3–36}$ [80]. PYY is released into the circulation in response to food intake [81], and $PYY_{3–36}$ is the predominant form of PYY in postprandial human plasma [80]. Its release is proportional to calorie intake, but is also influenced by meal composition, whereby fat intake stimulates higher plasma concentrations of PYY [81].

PYY shares 70% structural homology with neuropeptide Y (NPY). Whereas $PYY_{1–36}$ binds with high affinity to all NPY receptors, $PYY_{3–36}$ shows moderate selectivity for the NPY Y2 receptor. This receptor is an inhibitory presynaptic receptor that is highly expressed on NPY neurons, but barely present on neighboring proopiomelanocortin (POMC) neurons in the arcuate nucleus of hypothalamus (ARH) [26]. Indeed, only 3% of POMC neurons in the ARH express Y2 receptors [26,82].

Intraperitoneal administration of $PYY_{3–36}$ reduces food intake and body weight gain both in normal rodents [27] and in diverse rodent models of metabolic disease [83]. The anorectic effects of $PYY_{3–36}$ are also evident in humans. Two hours after receiving an infusion of $PYY_{3–36}$, volunteers with normal weight [27] and with obesity [84] were found to have reduced their food intake by more than 30% without a change in gastric emptying [27]. PYY acts centrally to inhibit gastric emptying and motility by a vagally mediated mechanism – the so-called 'ileal brake phenomenon' [33,85].

Although PYY is also expressed in the brain, its functions are unknown [86]. Because intracerebroventricular injection of PYY to sites other than the ARH strongly stimulates hyperphagia, the anorectic effects of PYY might depend on specific access to ARH neurons (and perhaps the area postrema).

on the presynaptic activation of NPY/AgRP neurons. In this manner, ghrelin functions like the neuropeptide $PYY_{3–36}$ (discussed below), but results in opposite effects. Thus, ghrelin directly activates orexigenic NPY/AgRP neurons and indirectly inhibits anorexigenic POMC neurons.

Recently, focus has been directed at elucidating the signal transduction mechanisms of ghrelin in ARH neurons. Using ratiometric fura-2 fluorescence imaging of isolated single ARH neurons, Kohno *et al.* [20] have shown that ghrelin dose-dependently increases cytosolic Ca^{2+} in a third of ARH neurons. Roughly 80% of these ghrelin-responsive neurons were found to be immunoreactive for NPY. Inhibitors of protein kinase A and N-type Ca^{2+} channels significantly attenuated the ghrelin-induced increases in cytosolic Ca^{2+}, suggesting that ghrelin directly interacts with NPY/AgRP neurons to induce Ca^{2+} signaling via protein kinase A and N-type Ca^{2+}-channel-dependent mechanisms [20]. These observations are partially consistent with recent electrophysiological evidence showing that ghrelin, as well as orexin (discussed below), produces a bursting pattern in NPY/AgRP neurons that is driven by low-threshold (T-type) Ca^{2+} conductances and transient outwardly rectifying K^+ conductances [13].

Both studies indicate that ghrelin induces increases in cytosolic Ca^{2+} in NPY/AgRP neurons in the ARH; however, the electrophysiology data demonstrate that ghrelin activates a low-voltage-activated T-type Ca^{2+} conductance, whereas the Ca^{2+} imaging data support the activation of N-type Ca^{2+} channels by ghrelin. The reasons for this discrepancy are unclear. In the electrophysiology experiments, T-type Ca^{2+} channels were found to be responsible for underlying membrane potential oscillations in a subtype of ARH neurons called ARH pacemaker neurons. These membrane oscillations were required for ghrelin-induced bursts of action potentials. The Ca^{2+} increases recorded in fura-2 imaging often result from neuronal depolarization and are therefore a good indicator of neuronal activation; however, it is not known what activity pattern ghrelin causes in these neurons. Thus, it is impossible to determine from the Ca^{2+} imaging data whether the activation of N-type Ca^{2+} channels by ghrelin occurred in the same subset of NPY/AgRP pacemaker neurons identified in the electrophysiology experiments [13].

Ghrelin in the brainstem

Although the ARH has been the principal focus of research on the central actions of ghrelin, the receptor for ghrelin – the growth hormone secretagogue receptor (GHS-R) – is expressed in the caudal brainstem [21], and administration of ghrelin directly into the dorsal vagal complex (DVC) produces hyperphagia with sensitivity comparable to that reported for the ARH [21]. The DVC, a hindbrain autonomic center that includes the nucleus tractus solitarius (NTS), dorsal motor nucleus of the vagus (DMV) and area postrema [22], functions as the central hub that provides parasympathetic control over the gastrointestinal tract and coordinates digestive functions. Perhaps surprisingly, then, peripheral injections of ghrelin (at doses that increase food intake) selectively induce c-Fos expression only in ARH neurons and not in other hypothalamic, limbic, pontine or medullary brain structures [23,24].

Although it is possible that peripheral ghrelin has inhibitory actions in the caudal brainstem (and thus would not be detected by c-Fos expression), there are no electrophysiological studies reporting the effect of ghrelin on hindbrain neurons. Nevertheless, recent evidence might curtail debate over whether the effect of ghrelin on food intake has a direct action on the hindbrain. Data from Chen *et al.* [19] firmly support the assertion that peripheral ghrelin stimulates feeding by acting through

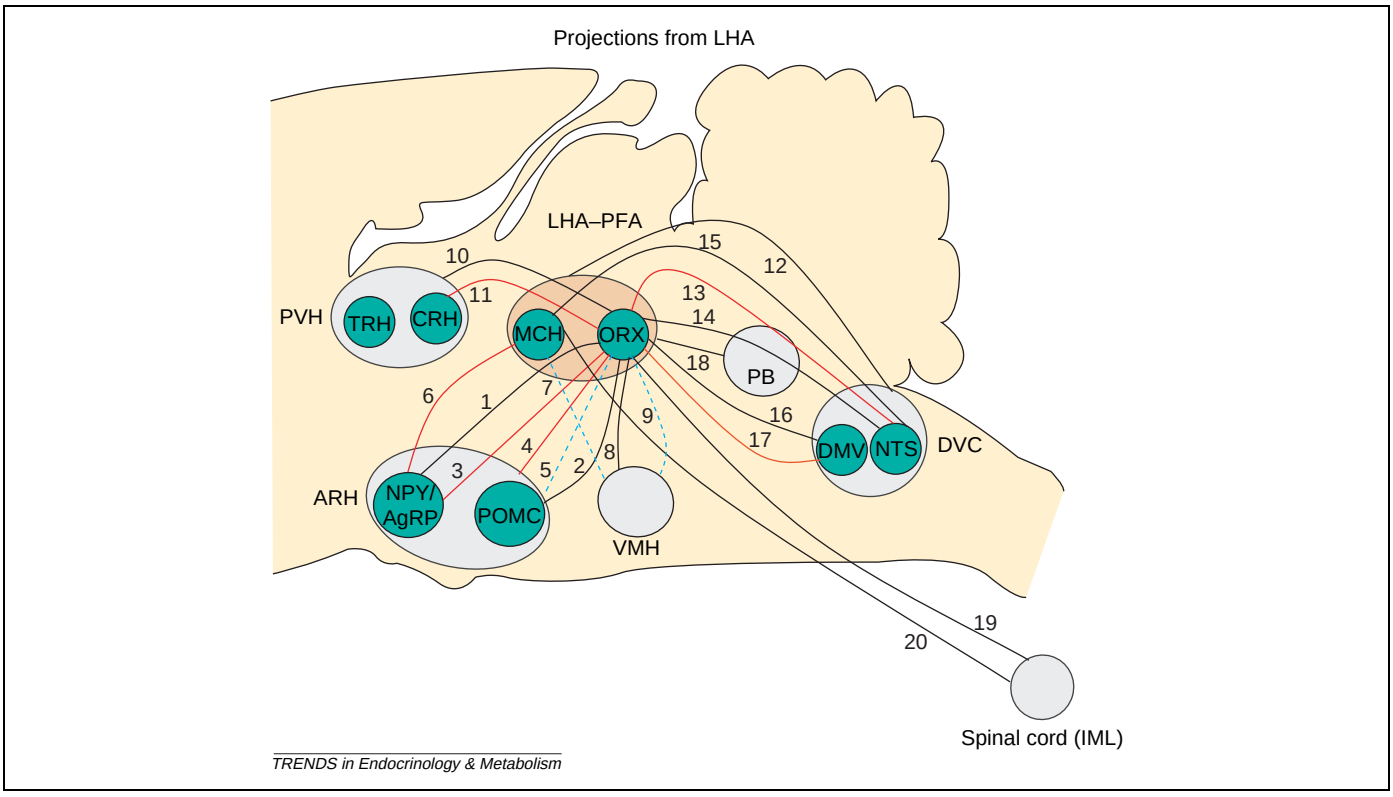

Figure 3. Projections from the lateral hypothalamic area (LHA). Shown are projections from the LHA to several hypothalamic nuclei including the arcuate nucleus of the hypothalamus (ARH), paraventricular nucleus of hypothalamus (PVH) and ventromedial nucleus of hypothalamus (VMH), and to extrahypothalamic areas such as the parabrachial nucleus (PB), dorsal vagal complex (DVC) and sympathetic preganglionic neurons in the spinal cord. Black lines show projections derived from anatomical studies, broken cyan lines show inhibitory inputs derived from physiological studies, and red lines show stimulatory inputs. Numbers to the left of each line refer to supporting references. LHA neurons transduce input from the ARH and other sources into changes in appetite, energy expenditure and neuroendocrine function. Orexin (ORX) and melanin-concentrating hormone (MCH) neurons in the LHA innervate the whole neuraxis. Orexin axons make asymmetric synapses on neurons expressing neuropeptide Y and agouti-related protein (NPY/AgRP) suggesting that orexin stimulates these neurons (1) [39,46]. ORX neurons make symmetrical synapses onto neurons expressing proopiomelanocortin (POMC), suggesting that ORX inhibits POMC neurons (2) [44]. Physiological studies support ultrastructural analyses (3) [13,42,43], (4) [41], (5) [43]. MCH projections to ARH neurons are excitatory in slices from overweight rats (6) [59]. By contrast, MCH projections to the VMH, a region that has been suggested to act as a satiety center, are inhibitory (7) [59]. Orexin neurons also project to the VMH (8) [47,95,108] and inhibit glucose-responsive neurons in the VMH (9) [43]. Orexin projections to parvocellular PVH neurons [putative corticotropin-releasing hormone (CRH) neurons] (10) [46,47] are excitatory (11) [111]. The LHA projects densely to the DVC, with most descending fibers terminating in the ipsilateral brain stem (12) [112]. Orexin projections to the nucleus of the solitary tract (NTS) (13) [88], (14) [46,47,114] are more abundant than those of MCH neurons to this region (15) [113]. In addition, orexin neurons have dense, primarily excitatory, projections to the dorsal motor nucleus of the vagus (DMV) (16) [46,114,115], (17) [116–119]. Finally, orexin neurons send projections to PB neurons (18) [47] and to preganglionic spinal cord neurons, (19) [115], whereas MCH neurons project to the spinal cord (20) [113].

ARH neurons. The primary discovery of this group is that mice lacking the genes encoding NPY and AgRP have no response to peripheral ghrelin-induced feeding. Crucially, Chen *et al.* have shown that ghrelin functions through NPY/AgRP neurons (in the ARH), and that ghrelin only works if NPY and AgRP are expressed, thereby identifying NPY/AgRP neurons as requisite mediators of ghrelin-stimulated feeding.

At face value these results seem difficult to reconcile with studies showing that administration of ghrelin directly into the brainstem produces hyperphagia [21], and that the blockade of afferent vagal neurotransmission or vagotomy prevents the response to peripheral ghrelin but has no effect on the feeding response to central administration of ghrelin [17,18]. One way to resolve the issue over whether brainstem circuitry is required for ghrelin-induced feeding would be to examine the extent of c-Fos expression that is induced in NPY/AgRP neurons by peripheral ghrelin in decerebrate animals. We anticipate that, if a ghrelin hindbrain relay is necessary for ghrelin-induced activation of NPY/AgRP neurons in the ARH, then decerebration, in which all brainstem to hypothalamic connectivity is severed, will prevent the c-Fos-mediated activation of NPY/AgRP neurons. At present, we can confidently state that the primary site of the central action of ghrelin is the ARH but we cannot rule out the possibility that it has a complementary action in the brainstem.

Electrophysiology of $PYY_{3–36}$

PYY in the ARH

Few studies have examined the central electrophysiological actions of the neuropeptide PYY (Box 2). The direct effects of leptin and ghrelin on ARH neurons suggest that these neurons might have access to circulating hormones, either via specific transport mechanisms or via diffusion from the median eminence – a circumventricular organ that has a more permeable blood–brain barrier [25].

On the basis of the differential distribution of the NPY Y2 receptor on ARH neurons [26], we and our colleagues [27] proposed that activation of the Y2 receptor by $PYY_{3–36}$ would inhibit NPY/AgRP neurons and thereby disinhibit POMC neurons. In recordings from the ARH in hypothalamic slices, we found that $PYY_{3–36}$ depolarized POMC neurons and increased the frequency of spontaneous

Box 3. Central mediators of energy homeostasis

Orexin

The orexins (also known as the hypocretins) comprise a pair of peptides, orexin A and orexin B, that are derived from the same precursor peptide (prepro-orexin) by proteolytic processing. The orexins are expressed by a population of neurons in the lateral hypothalamic area (LHA) and perifornical area [34], and to a lesser extent in the dorsomedial nucleus of the hypothalamus (DMH) [39].

The orexins bind and activate two closely related G-protein-coupled receptors, termed OX1R and OX2R. OX1R is relatively selective for orexin A, whereas OX2R is a nonselective receptor for both orexin A and orexin B [35]. Considerable evidence suggests that OX1R is coupled to activation of the G_q subtype of GTP-binding proteins, leading to stimulation of phospholipase C and protein kinase C [87,88]. OX2R might be coupled to G_q proteins in cultured pituitary somatotropes [89], and recent evidence suggests that this receptor is coupled to pertussis-toxin-sensitive G_i-and/or G_o-binding proteins in proopiomelanocortin (POMC) neurons in the arcuate nucleus of hypothalamus (ARH) [43].

In contrast to the fairly restricted distribution of orexin-immunoreactive somas, the projection of orexinergic fibers and the expression of orexin receptors are extensively distributed throughout the cerebral cortex, limbic system, posterior hypothalamus, thalamus and brainstem (Figure 3) [34,46,47,90]. OX1R and OX2R show, however, a markedly different distribution [91,92]. In the hypothalamus, expression of OX1R mRNA is largely restricted to the ventromedial nucleus of hypothalamus (VMH) and DMH, whereas expression of OX2R mRNA is high in the paraventricular nucleus of hypothalamus (PVH), VMH and ARH, as well as in the mammillary nuclei [92].

When administered centrally, orexin increases feeding, locomotion and wakefulness [35,93,94], consistent with activation of c-Fos in areas mediating these actions [46]. By contrast, fasting increases the levels of prepro-orexin mRNA [35].

Melanin-concentrating hormone

Melanin-concentrating hormone (MCH) is synthesized primarily in neurons in the LHA [49], but in a population that is distinct from the one that produces orexin [95]. MCH binds to a G-protein-coupled receptor termed either somatostatin-like (SLC-1) or MCH-1R [52]. Humans also express a second receptor for MCH, MCH-2R, that is not present in the rodent brain [96]. Expression of MCH-1R is widespread in the brain. In the hypothalamus, MCH-1R is found in VMH, ARH and DMH hypothalamic nuclei [52,53,58], as well as in the LHA [58]. In humans, MCH-2R is densely expressed in the ARH and the VMH [97].

Similar to orexin, MCH is an important regulator of feeding behavior [98]. Central administration of MCH stimulates food intake, whereas fasting increases MCH expression [98]. MCH-deficient mice have lower body weights owing to reduced feeding and enhanced metabolism, despite their reduction in both leptin and POMC mRNA in the ARH [99]. Conversely, transgenic overexpression of MCH in the LHA increases the body weight of mice fed on standard and high-fat diets [100].

Notably, both orexin and MCH in the LHA might be involved in communicating the hedonic or rewarding aspects of feeding [6,101]. Feeding is powerfully influenced by pleasure and reward [6], and the LHA is involved in feeding, arousal and motivated behaviors [101]. LHA neurons are well placed for a role in the reward pathway. Orexin receptors are expressed at high levels in limbic regions such as the ventral tegmental area and the locus coeruleus [92,102]. Likewise, it is possible that MCH signaling from the LHA to the nucleus accumbens, a principal reward region in the brain, is involved in hedonic aspects of feeding.

action potentials [27]. In addition, $PYY_{3–36}$ decreased the frequency of inhibitory postsynaptic currents onto POMC neurons. An agonist specific for the Y2 receptor produced similar results, consistent with the hypothesis that activation of the Y2 receptor on NPY nerve terminals activates POMC neurons by decreasing the release of NPY and GABA onto POMC neurons [27]. This finding is also in agreement with the *in vivo* observation that peripheral injections of $PYY_{3–36}$ do not inhibit feeding in Y2 receptor knockout mice [27].

Currently, we cannot state that the effects of PYY on POMC neurons are exclusively due to the inhibition of apposing NPY/AgRP neurons. Because only a few POMC neurons in the ARH express Y2 receptors [26], it is likely that the activation of POMC neurons by PYY is primarily a presynaptic effect.

PYY in the brainstem

Binding sites with affinity for PYY, specifically Y1 and Y2 receptors, have been identified in the DVC [28,29], which suggests that this region might have access to PYY *in vivo*. A portion of the DVC (neurons in the area postrema) lies outside the blood–brain barrier, and these neurons might respond to circulating gastrointestinal hormones and relay these signals to the NTS and parabrachial nucleus [30].

Using whole-cell patch clamp recordings from identified GI-projecting neurons of the rat DMV, Browning and Travagli [31] have shown that the main effect of $PYY_{3–36}$ in the DVC is an inhibition of glutamatergic synaptic transmission between the NTS and the DMV. This PYY-induced decrease in excitatory transmission between the NTS and the DMV results from activation of presynaptic Y2 receptors. These results are consistent with earlier studies showing that PYY-mediated inhibitory actions on gastrointestinal motility result from interactions with brainstem Y2 receptors [32,33].

Thus, the effects of circulating $PYY_{3–36}$ after ingestion of a meal seem to be mediated by the Y2 receptor in both the brainstem and the ARH. Notably, the same peptide suppresses digestive functions by decreasing 'excitatory' tone in the DVC and suppresses appetite by decreasing 'inhibitory' tone in the ARH.

Electrophysiology of orexin

Orexin in the lateral hypothalamic area

Owing to the widespread distribution of orexin-immunoreactive axons (Figure 3), the actions of orexin have been investigated in several feeding-related regions of the CNS (Box 3). So far, orexin action has been found to be excitatory in all of the regions analyzed with the notable exception of the ARH.

The earliest studies on cultured neurons showed that orexin was excitatory to hypothalamic neurons, but not to hippocampal neurons [34]. In hypothalamic slices, the effects of orexin were first assessed in the lateral hypothalamic area (LHA) itself. The LHA contains orexin neurons and glucose-sensitive neurons (GSNs), both of which are stimulated by hypoglycemia and are implicated in hypoglycemia-induced feeding [35,36]. When applied to hypothalamic brain slices, orexin A was found to increase spontaneous action potentials by 500% and to cause a rapid, long-lasting tetrodotoxin-resistant depolarization of glucose-sensitive neurons, indicating that it has a direct postsynaptic effect on GSNs in the LHA [37]. Confocal microscopic evaluation has shown that orexin-immunoreactive axons are often intertwined with GSN dendrites, establishing putatively synaptic contacts [37].

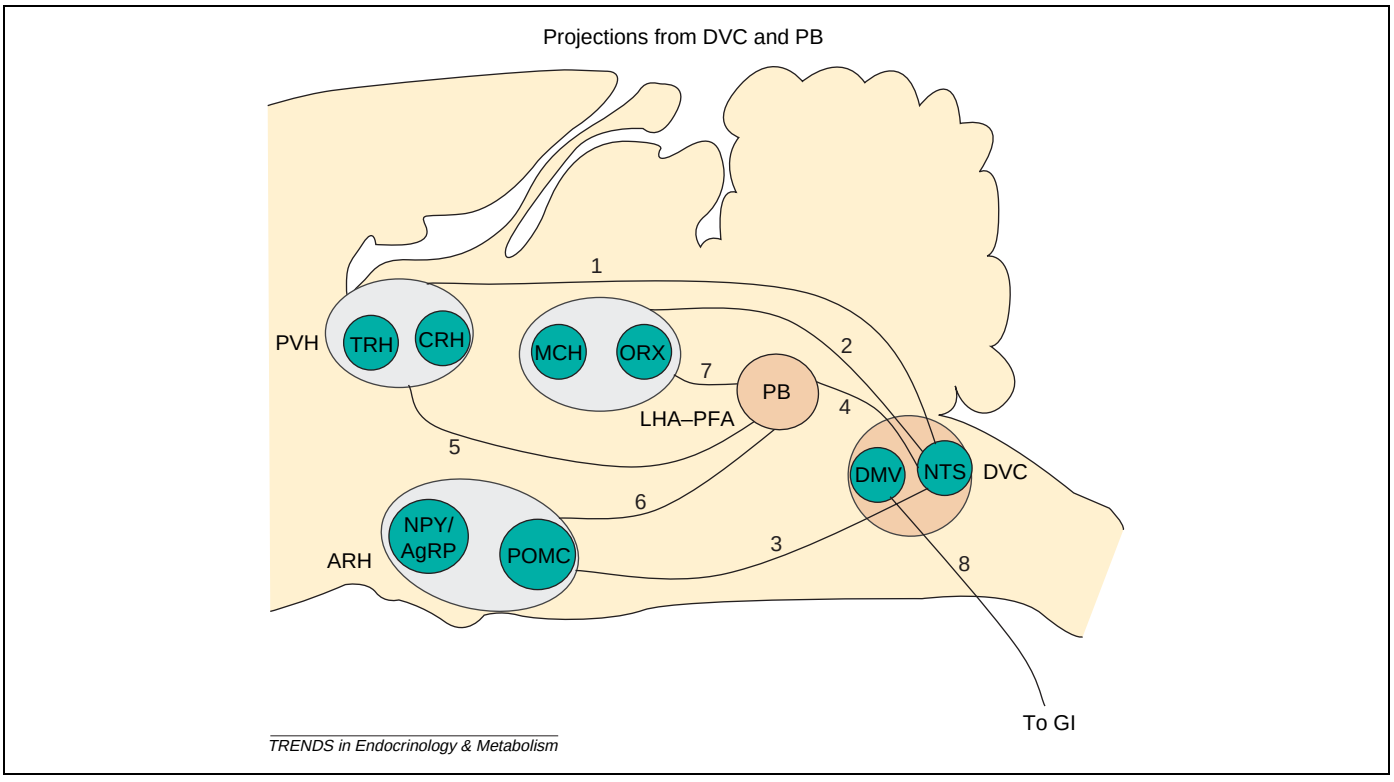

Figure 4. Projections from dorsal vagal complex (DVC) and parabrachial nucleus (PB). Shown are projections from the DVC and PB to several hypothalamic nuclei including the paraventricular nucleus of hypothalamus (PVH), lateral hypothalamic area (LHA) and ventromedial nucleus of hypothalamus (VMH), and to extrahypothalamic areas such as sympathetic preganglionic neurons in the spinal cord. Black lines show projections derived from anatomical studies. The nucleus of the solitary tract (NTS) projects to several hypothalamic nuclei including the PVH (1) [120,121], LHA (2) [120,121] and arcuate nucleus of hypothalamus (ARH) (3) [120,121]. In addition, the caudal NTS has ascending projections to innervate the PB (4) [6,120]. Direct projections from the PB also innervate the PVH (5) [120–122], ARH (6) [122] and LHA (7) [122]. Vagal afferent fibers from the gastrointestinal (GI) tract form synapses in the NTS, and in turn project heavily onto preganglionic motor neurons in the dorsal motor nucleus of the vagus (DMV), which are a chief source of parasympathetic innervation to the subdiaphragmatic visceral organs. Efferent parasympathetic visceromotor fibers from the DMV innervate the whole gastrointestinal tract (8) [116,123,124]. Modulation of the vagovagal circuits in the DVC by descending inputs from the hypothalamic nuclei constitutes the primary means by which higher centers exert a direct influence over gastric and pancreatic secretion, and stomach and small intestinal motility. Abbreviations: CRH, corticotrophin-releasing hormone; MCH, melanin-concentrating hormone; NPY/AgRP, neuropeptide Y/agouti-related protein; ORX, orexin; PFA, perifornical area; POMC, proopiomelanocortin; TRH, thyrotropin-releasing hormone.

To assess the response of orexin neurons to feeding-related signals, Yamanaka *et al.* [38] generated transgenic mice expressing a fusion protein of orexin and enhanced green fluorescent protein (orexin–EGFP). In dispersed cultures and in hypothalamic slices, orexin–EGFP neurons depolarized in response to glutamate and ghrelin (a peripheral appetite-stimulating peptide) and hyperpolarized in response to GABA and leptin (a peripheral appetite-inhibiting hormone). Similarly, orexin–EGFP neurons had a predictable response to changes in extracellular glucose concentration; namely, they depolarized in response to falling levels and hyperpolarized in response to rising levels. Notably, insulin had no effect on orexin–EGFP neurons [38].

Thus, both the direct responsiveness of orexin neurons to feeding-related signals and the subsequent activation of GSNs by orexin might mediate the onset of hypoglycemia-induced feeding and contribute to the appetite-stimulating effect of orexin A.

Orexin in the ARH and VMH

Given that orexin-immunoreactive axon terminals are exceptionally abundant in the ARH [39] and that the orexin receptor OX1R is located in both POMC- and NPY-expressing neurons in the ARH [40], several studies have evaluated the electrophysiological effects of orexin in the ARH [13,41,42]. Early extracellular recordings showed that orexin increases the firing rate of most ARH neurons [42]. Recently, van den Top *et al.* [13] assessed the effect of orexin on the activity 'pattern' of NPY/AgRP neurons in the ARH. Importantly, these investigators noted that the application of orexin, as well as ghrelin (see above), produced bursts of action potentials corresponding to underlying membrane potential oscillations. This bursting pattern, driven by low-threshold Ca^{2+} conductances and transient outwardly rectifying potassium conductances [13], has been shown to be a more effective stimulus for neuropeptide release than has repetitive firing [9]. Crucially, the effects of orexin on GABAergic NPY/AgRP neurons would preferentially release the two potent orexigens NPY and AgRP.

Using similar techniques, Burdakov *et al.* [41] have also reported that a subset of GABAergic ARH neurons is directly activated by orexins. They found that the concentration dependency of this activation was similar for orexin A and B, suggesting involvement of the orexin receptor OX2R. Neuronal activation by orexins was dependent on the mobilization of Ca^{2+} from intracellular stores, which Burdakov *et al.* concluded was dependent on an Na^{+}/Ca^{2+} exchanger. Although this conclusion is one potential interpretation, it was based on data showing that an Na^{+}/Ca^{2+} inhibitor reversed the actions of orexin.

Because current–voltage relationships before and after the addition of the pump inhibitor were not shown, however, at present we are not convinced that this is the mechanism by which orexin activates NPY neurons in the ARH.

What about the effects of orexin on POMC neurons in the ARH? Similar to their synaptic contacts onto NPY/AgRP neurons in the ARH [39,43], orexin fibers also make synaptic contacts onto POMC neurons [43,44]. These orexin–POMC synapses are primarily symmetrical at the ultrastructural level, which suggests that orexin inhibits POMC neurons [44]. Although no electrophysiological evidence supports this presumption or assertion, it is consistent with the rationale that an orexinergic peptide would inhibit the activity of POMC neurons expressing the anorexigenic peptide α-MSH.

Contrary to previous reports that orexin shows only excitatory properties, data from Ca^{2+} imaging suggest that orexin has both excitatory and inhibitory roles in the ARH. Using fura-2 fluorescence imaging of isolated single ARH neurons to assess changes in cytosolic Ca^{2+}, Muroya *et al.* [43] have shown that physiological doses of orexin A increase cytosolic Ca^{2+} in NPY neurons, consistent with other data [13,41]. In addition, they found that orexin decreases cytosolic Ca^{2+} in POMC neurons. Orexin increases cytosolic Ca^{2+} biphasically, with pharmacological properties consistent with mediation by OX1R, phospholipase C, inositol (1,4,5)-trisphosphate and protein kinase C signaling pathways. By contrast, the orexin-mediated decrease in cytosolic Ca^{2+} in POMC neurons is most probably mediated by OX2R (given the comparable responses to orexin A and orexin B) and involves the activation of G_i and/or G_o GTP-binding proteins (given the sensitivity to pertussis toxin). Future electrophysiological recordings of the effects of orexin on POMC neurons in hypothalamic slice preparations will be helpful to corroborate these Ca^{2+} imaging data.

Shiraishi *et al.* [45] have compared the effects of orexin and leptin on extracellular neuronal activity in the VMH. They found that orexin significantly decreases the activity of glucoresponsive neurons (GRNs) – neurons that are normally excited by rising glucose concentrations. Conversely, leptin increases the activity of GRNs in comparison to non-GRNs. These data have been corroborated by Ca^{2+} imaging data showing the reciprocal effects of these peptides on cytosolic Ca^{2+}; namely, orexin decreases cytosolic Ca^{2+} and leptin increases cytosolic Ca^{2+} in GRNs in the VMH [43]. Thus, orexins might stimulate feeding by acting in the ARH to activate NPY/AgRP neurons and to inhibit POMC neurons, while simultaneously inhibiting GRNs in the VMH. At this stage, future experiments in the ARH and VMH must be done to complete the emerging picture of how leptin and orexin act reciprocally to mediate feeding status.

Orexin in the brainstem

The NTS is a brainstem region that has a central role in many autonomic functions and receives dense orexin innervation from the LHA [46,47]. Indeed, orexin A has been found to depolarize more than 90% of NTS neurons tested, by concurrently inhibiting a specific K^+ conductance and activating a nonselective cationic conductance [48]. The NTS sends efferent output to many hypothalamic nuclei as well as to the DMV, whose efferent parasympathetic visceromotor fibers innervate the whole gastrointestinal tract (Figure 4). Whether the actions of orexin in the NTS have a direct role in feeding regulation is, however, currently unknown.

Electrophysiology of melanin-concentrating hormone

Melanin-concentrating hormone in the LHA

The highest density of melanin-concentrating hormone (MCH)-immunoreactive axons is found in the LHA, and many of these axons make synaptic contacts with other LHA neurons [49]. In cultured synaptically coupled LHA neurons, MCH potently inhibits synaptic activity (Box 3) [50]. Although MCH has no direct effect on resting membrane potential of LHA neurons, its application reduces and sometimes abolishes spontaneous action potentials. Notably, Gao and van den Pol [50] have suggested that the mechanism underlying the inhibition of spontaneous firing by MCH might be related to an indirect effect on neurotransmission because synaptic transmission mediated by glutamate (and GABA to a lesser extent) is inhibited by MCH.

A powerful mechanism for controlling neurotransmitter release is the modulation of Ca^{2+} influx via voltage-dependent Ca^{2+} channels at presynaptic terminals [51]. In a subsequent study, Chen and van den Pol [51] showed that the inhibitory action of MCH in LHA neurons is due to the inhibition of voltage-dependent Ca^{2+} currents (with L- and N-type channels accounting for most of the voltage-activated current) via G-protein pathways that are sensitive to pertussis toxin [51].

MCH in the ARH and VMH

Given the density of both MCH-immunoreactive fibers [49] and the MCH receptor MCH-1R [52,53] in the VMH and ARH, Davidowa *et al.* [54] studied the effect of MCH on single-unit activity in hypothalamic slices containing the VMH and ARH in normal and overweight rats. In slices from normally fed or food-deprived rats of normal weight, 20–40% of tested neurons responded to the application of MCH, showing either an increase or decrease in spontaneous activity. By contrast, MCH produced consistent responses in neurons from overweight rats: it predominantly activated ARH neurons and inhibited VMH neurons.

Davidowa *et al.* [54] proposed that MCH supports a shift in energy balance to a higher level of body weight in overweight animals by simultaneously activating NPY neurons in the ARH (to promote feeding) and inhibiting neurons in the VMH (to reduce energy expenditure) [54]. The signaling mechanisms that promote or facilitate this switch in energy balance are completely unknown. It is also worthwhile noting that the MCH-activated neurons were identified as NPY neurons solely by their location in the ARH and not by immunocytochemical characterization.

MCH in the paraventricular nucleus of hypothalamus

The paraventricular nucleus of hypothalamus (PVH), in particular its parvicellular region, is involved in regulating body weight [55,56]. This region has long been

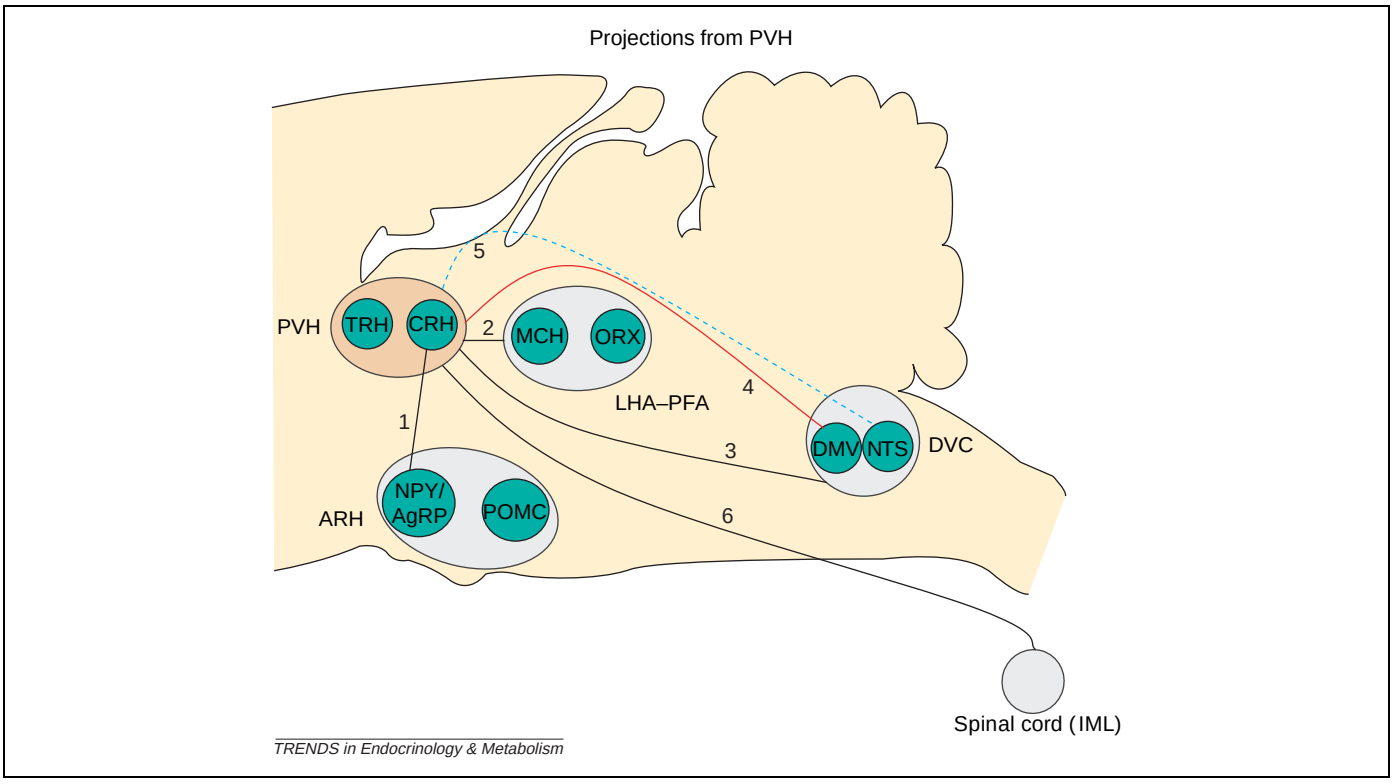

Figure 5. Projections from the paraventricular nucleus of hypothalamus. Shown is a summary of the main projections from the PVH to various hypothalamic nuclei including the lateral hypothalamic area (LHA) and ventromedial nucleus of hypothalamus (VMH), and to extrahypothalamic areas such as the parabrachial nucleus (PB), dorsal vagal complex (DVC) and sympathetic preganglionic neurons in the spinal cord. Black lines show projections derived from anatomical studies, the broken cyan line inhibitory inputs derived from physiological studies, and the red line shows stimulatory inputs. Numbers to the left of each line refer to supporting references. The PVH is an integrating center on which many neural pathways that influence energy homeostasis converge. The ventromedial portion of the arcuate nucleus of hypothalamus (ARH), an area containing neurons expressing neuropeptide Y and agouti-related protein (NPY/AgRP), shows corticotrophin-releasing hormone (CRH) receptor binding and CRH receptor mRNA. Although this suggests that there is possible reciprocal feedback regulation between NPY/AgRP and CRH neurons, CRH fiber innervation to the ARH is extremely sparse (1) [125]. The PVH has only minor reciprocal projections to the LHA and VMH (2) [126]. Retrograde and anterograde tracing studies have demonstrated a substantial projection from the PVH to the DVC (3) [112,127,128]. These projections are primarily excitatory in the dorsal motor nucleus of the vagus (DMV) (4) [117,118]. By contrast, most neurons in the nucleus of the solitary tract (NTS) are inhibited by PVH stimulation (5) [118]. The PVH also provides a major descending projection to autonomic preganglionic neurons in the medulla and spinal cord (6) [127,129].

identified as a 'satiety center', because lesions within it produce hyperphagic obesity [57].

The MCH receptor MCH-1R is abundantly expressed in the PVH [58], and Davidowa *et al.* [59] have studied the effect of MCH on single-unit activity in hypothalamic slices containing the PVH, mainly its medial parvicellular part, from normal and overweight rats. In slices from rats of normal weight, the application of MCH did not have a predictable response and excited or inhibited equal numbers of neurons. By contrast, neurons from overweight rats were predominantly inhibited by MCH [59]. Because the output from the PVH is predominantly catabolic (Figure 5) [60], we anticipate that an inhibition of PVH neurons by MCH would continue to support the already existing positive energy balance in overweight rats. The signaling pathways in normal and overweight animals need to be examined further to understand how states in energy balance can change the effects of a single neuropeptide.

Future directions

Our understanding of feeding circuits from electrophysiology experiments has led to a working circuit diagram including several nuclei in the hypothalamus and brainstem. Our goal is to use this circuit as a guide to test putative feeding-related signals and potential therapeutic drugs at relevant target sites. Future experiments need to test the convergence of multiple feeding-related signals, because most studies have assessed the effects of single agents on neuronal activity and on energy homeostasis. The development of models that facilitate recording from specific cell types, coupled with tracing studies, will accelerate the mapping of these circuits. It is also important to test the function of these circuits in altered physiological states – for example, in obese animals and animals that are becoming obese. As we learn more, we will no doubt expand and challenge our current understanding of feeding circuits.

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